RESEARCH NOTE

Evaluation of the CLSI cefoxitin 30-μg disk-diffusion method for detecting methicillin resistance in staphylococci

L.-X. Zhu[1,2,3], Z.-W. Zhang[3], C. Wang[3], H.-W. Yang[3], Q. Zhang[3] and J. Cheng[1,2,3,4]

[1]Department of Biological Sciences and Biotechnology, [2]Medical Systems Biology Research Center, [3]The State Key Laboratory of Biomembrane and Membrane Biotechnology, Tsinghua University, and [4]National Engineering Research Centre for Beijing Biochip Technology, Beijing, China

ABSTRACT

Methicillin susceptibility of 415 staphylococcal isolates from Chinese hospitals was assessed using the CLSI disk-diffusion method with a cefoxitin 30-μg disk in comparison with an oxacillin 1-μg disk. PCR-based detection of *mecA* was the reference standard. The cefoxitin 30-μg disk performed with almost the same high level of accuracy as the oxacillin 1-μg disk in detecting methicillin resistance in *Staphylococcus aureus*. For coagulase–negative staphylococci (CoNS), the sensitivity of the cefoxitin 30-μg disk was 90.5%, compared with 83.4% for the oxacillin 1-μg disk. Confirmatory testing of isolates with borderline susceptibility and revision of the cefoxitin breakpoint are proposed in order to categorise CoNS more accurately.

Keywords Cefoxitin disk, coagulase-negative staphylococci, *mecA* gene, methicillin resistance, PCR, susceptibility testing

Original Submission: 7 January 2006; **Revised Submission:** 24 February 2006; **Accepted:** 27 March 2006

Clin Microbiol Infect 2006; 12: 1039–1042
10.1111/j.1469-0691.2006.01522.x

Corresponding author and reprint requests: J. Cheng, Department of Biological Sciences and Biotechnology, Tsinghua University, Beijing 100084, China
E–mail: jcheng@mail.tsinghua.edu.cn

Penicillin-binding protein (PBP) 2a, encoded by the *mecA* gene, is the main cause of methicillin resistance in staphylococci [1]. Since methicillin-resistant staphylococci have become widely prevalent, the glycopeptide antibiotic vancomycin has become the most common treatment for staphylococcal infections. The emergence of *Staphylococcus aureus* and coagulase-negative staphylococci (CoNS) with resistance to glycopeptides emphasises the importance of the prudent use of antibiotics and the need for reliable laboratory identification of methicillin-resistant strains [2,3]. A variety of testing methods for the detection of methicillin-resistant *S. aureus* (MRSA) have been developed [4–8]. However, the reliability of methicillin susceptibility testing for CoNS isolates is currently a major concern because of the more heterogeneous expression of the *mecA* gene [9]. Use of a cefoxitin disk has recently been proposed as an alternative to an oxacillin disk for detecting methicillin resistance in staphylococci [10–17]. In the present study, tests using cefoxitin 30-μg disks were compared to tests using oxacillin 1-μg disks by the CLSI (formerly NCCLS) method [18]. PCR-based detection of *mecA* was used as the reference standard.

In total, 415 epidemiologically unrelated clinically significant isolates (178 *S. aureus* and 237 CoNS) were recovered (January–May 2003) from hospitalised patients in all departments, including the intensive care units, of several hospitals in China (Beijing Hospital, Beijing Tiantan Hospital, Beijing Tongren Hospital and Peking Union Medical College Hospital). Duplicate samples from the same patient were excluded. Methicillin-resistant isolates were selected on the basis of oxacillin resistance, as determined by the CLSI disk-diffusion method [18], and methicillin-susceptible isolates were selected randomly. Among the isolates, 145 (34.9%) were from blood, 110 (26.5%) were from pus, 92 (22.2%) were from the respiratory tract, and 44 (10.6%) were from urine. All the *S. aureus* and CoNS isolates were identified to the species level with the Vitek 2 instrument (bioMérieux, Rouen, France). For CoNS, the collection comprised 144 *Staphylococcus epidermidis*, 41 *Staphylococcus haemolyticus*, 18 *Staphylococcus auricularis*, 13 *Staphylococcus simulans*, seven *Staphylococcus hominis*, four *Staphylococcus capitis*, four *Staphylococcus sciuri* and six unidentified CoNS isolates.

Antimicrobial susceptibility was tested by the CLSI disk-diffusion method [18] on Müeller–Hinton agar with oxacillin 1-μg and cefoxitin 30-μg disks (Tiantan Biotechnology Co., Beijing, China). Plates were incubated for 24 h at 35°C before measuring inhibition zone diameters. *S. aureus* ATCC 25923 was included as a control strain; inhibition zone diameters with oxacillin 1-μg disks and cefoxitin 30-μg disks were within the published limits (19–24 mm and 23–29 mm, respectively). PCR for the *mecA* gene used primers mecA-f (5′- GATGGCTATCGTGTCACAATC) and mecA-r (5′-TGAGTTGAACCTGGTGAAGT) to generate a 352-bp amplicon. Of the 178 *S. aureus* isolates, 119 were *mecA*-positive and 59 were *mecA*-negative. Of the 237 CoNS, 199 were *mecA*-positive and 38 were *mecA*-negative. All the PCR products were further validated by hybridisation using gene-specific probes (results not shown).

The relationship between the disk-diffusion results and *mecA* status is summarised in Table 1. The current CLSI breakpoints for cefoxitin and oxacillin both separated the resistant and susceptible populations of *S. aureus* with satisfactory accuracy, as reported previously [10–12]. The sensitivities of the oxacillin 1-μg disk and the cefoxitin 30-μg disk tests were 97.5% and 96.6%, and the specificities were 98.3% and 94.9%, respectively. With most isolates, the inhibition zone diameters for *mecA*-positive and *mecA*-negative *S. aureus* were distinct with both agents. The oxacillin zone diameters for *mecA*-positive *S. aureus* were 6–10 mm, and for *mecA*-negative *S. aureus* were 15–30 mm, except for three false-susceptible isolates (zone diameters 14, 15 and 16 mm, respectively) and one false-resistant isolate (zone diameter 6 mm). The cefoxitin zone diameters for *mecA*-positive *S. aureus* were 6–19 mm and for *mecA*-negative *S. aureus* were 20–32 mm, except for four false-susceptible isolates (zone diameters 20 mm for three and 23 mm for one) and three false-resistant isolates (zone diameters 6 mm for two and 19 mm for one). One *mecA*-positive and one *mecA*-negative *S. aureus* gave discrepant results with both oxacillin and cefoxitin. There was no obvious explanation for the *mecA*-negative isolates with no zone of inhibition to oxacillin or cefoxitin, but the results were reproducible. In addition, three of six false-susceptible *S. aureus* isolates were shown by RAPD analysis to be related genotypically (data not shown).

With the 237 CoNS isolates, the cefoxitin 30-μg disk test (sensitivity 90.5%) was significantly more accurate ($p < 0.05$ by Mann–Whitney *U*-test) than the oxacillin 1-μg disk test (sensitivity 83.4%). Numerous discrepancies were seen, particularly among *mecA*-positive, oxacillin-susceptible or cefoxitin-susceptible isolates (Fig. 1). Among 199 *mecA*-positive CoNS isolates, 19 (9.5%) with distinct RAPD patterns were classified falsely as susceptible in the cefoxitin test. In the present study, the sensitivity of the tests with CoNS was lower than that reported by Swenson *et al*. [15]. The inhibition zone diameters of many isolates were within 1 mm of the breakpoints (Fig. 1), resulting in poor separation of the resistant and susceptible populations, and conse-

Table 1. Detection of methicillin resistance in 415 clinical isolates of staphylococci by disk-diffusion with oxacillin and cefoxitin disks

Organism	Agent tested	Susceptibility by disk-diffusion	Number of isolates		Sensitivity[a] (%)	Specificity[b] (%)
			mecA +	*mecA* –		
Staphylococcus aureus			*n* = 119	*n* = 59		
	Oxacillin	R	116	1[c]	97.5	98.3
		S	3[c]	58		
	Cefoxitin	R	115	3[c]	96.6	94.9
		S	4[c]	56		
CoNS			*n* = 199	*n* = 38		
	Oxacillin	R	166	9[c]	83.4	76.3
		S	33[c]	29		
	Cefoxitin	R (CLSI)	180	8[c]	90.5/97.0	78.9/71.1
		R (proposed breakpoints)[d]	193	11[c]		
		S	19[c]	30		
		S (proposed breakpoints)[d]	6[c]	27		

R, resistant; S, susceptible; CoNS, coagulase-negative staphylococci.
[a]Sensitivity was calculated as the number of isolates with true-positive results/the number of *mecA*-positive isolates.
[b]Specificity was calculated as the number of isolates with true-negative results/the number of *mecA*-negative isolates.
[c]Indicates isolates with a discrepancy between the phenotypic test and the *mecA* status determined by PCR.
[d]S = 27 mm; R = 26 mm.

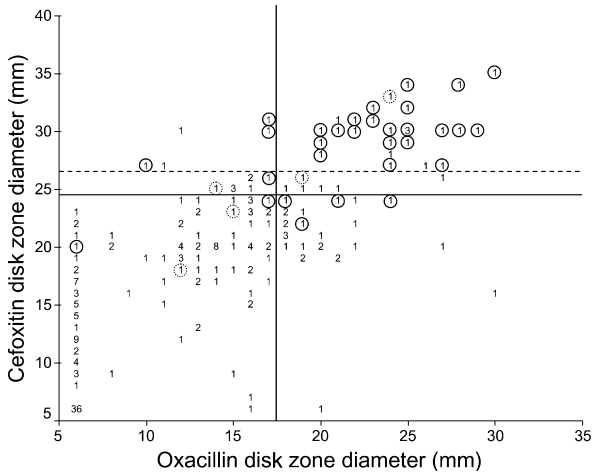

Fig. 1. Scattergram of inhibition zone diameters obtained using cefoxitin 30-μg and oxacillin 1-μg disks for 237 CoNS isolates. Unbroken circles indicate *mecA*-negative isolates; broken circles indicate one *mecA*-negative and one *mecA*-positive isolate at that position. Unbroken lines indicate the current CLSI interpretive criteria for CoNS; the broken line indicates a proposed breakpoint for cefoxitin.

quently, some incorrect results (13 of the isolates with zones within 1 mm of the breakpoint for each agent). Furthermore, of the 13 *mecA*-negative CoNS isolates that were categorised as resistant by either the cefoxitin or the oxacillin test, MICs of oxacillin, determined by the CLSI agar dilution method [19], were 0.5 to >16 mg/L for 12 isolates, indicating that resistance mechanisms other than that mediated by *mecA* were present.

In order to improve the reliability of oxacillin and cefoxitin disk-diffusion tests using the CLSI method, a PCR test for *mecA* or a latex agglutination test for PBP2a should be performed on any CoNS isolate with an oxacillin zone diameter of 17–27 mm, or a cefoxitin zone diameter of 24–31 mm. As suggested previously [17], the present data also indicate that breakpoints of S ≥27 mm and R ≤26 mm for the cefoxitin 30-μg disk would provide a high sensitivity (97.0%) with CoNS, but at the expense of specificity (71.1%).

ACKNOWLEDGEMENTS

This work was funded by a grant from the National Hi-Tech Program of China (No. 2002AA2Z2011) from the Department of Science and Technology, China. The authors wish to thank W.-P. Yang, X.-X. Zhu and K. Mitchelson for their critical reading of this manuscript. We thank T.-T. Cai, D. Jiang, X. Zhou, Q.-M. Ma, N. Du and N. Zhang for their proficient technical support.

REFERENCES

1. Hartman BJ, Tomasz A. Low-affinity penicillin-binding protein associated with beta-lactam resistance in *Staphylococcus aureus*. *J Bacteriol* 1984; **158**: 513–516.
2. Hiramatsu K, Hanaki H, Ino T, Yabuta K, Oguri T, Tenover FC. Methicillin-resistant *Staphylococcus aureus* clinical strain with reduced vancomycin susceptibility. *J Antimicrob Chemother* 1997; **40**: 135–136.
3. Chang S, Sievert DM, Hageman JC *et al.* Brief report: infection with vancomycin-resistant *Staphylococcus aureus* containing the *vanA* resistance gene. *N Engl J Med* 2003; **348**: 1342–1347.
4. van Griethuysen A, Pouw M, van Leeuwen N *et al*. Rapid slide latex agglutination test for detection of methicillin resistance in *Staphylococcus aureus*. *J Clin Microbiol* 1999; **37**: 2789–2792.
5. Hussain Z, Stoakes L, Garrow S, Longo S, Fitzgerald V, Lannigan R. Rapid detection of *mecA*-positive and *mecA*-negative coagulase-negative staphylococci by an anti-penicillin binding protein 2a slide latex agglutination test. *J Clin Microbiol* 2000; **38**: 2051–2054.
6. Louie L, Matsumura SO, Choi E, Louie M, Simor AE. Evaluation of three rapid methods for detection of methicillin resistance in *Staphylococcus aureus*. *J Clin Microbiol* 2000; **38**: 2170–2173.
7. Smyth RW, Kahlmeter G. Mannitol salt agar–cefoxitin combination as a screening medium for methicillin-resistant *Staphylococcus aureus*. *J Clin Microbiol* 2005; **43**: 3797–3799.
8. Kohner P, Uhl J, Kolbert C, Persing D, Cockerill F. Comparison of susceptibility testing methods with *mecA* gene analysis for determining oxacillin (methicillin) resistance in clinical isolates of *Staphylococcus aureus* and coagulase-negative *Staphylococcus* spp. *J Clin Microbiol* 1999; **37**: 2952–2961.
9. Chambers HF. Methicillin resistance in staphylococci: molecular and biochemical basis and clinical implications. *Clin Microbiol Rev* 1997; **10**: 781–791.
10. Felten A, Grandry B, Lagrange PH, Casin I. Evaluation of three techniques for detection of low-level methicillin-resistant *Staphylococcus aureus* (MRSA): a disk diffusion method with cefoxitin and moxalactam, the Vitek 2 system, and the MRSA-screen latex agglutination test. *J Clin Microbiol* 2002; **40**: 2766–2771.
11. Skov R, Smyth R, Clausen M *et al*. Evaluation of a cefoxitin 30 μg disc on iso-sensitest agar for detection of methicillin-resistant *Staphylococcus aureus*. *J Antimicrob Chemother* 2003; **52**: 204–207.
12. Cauwelier B, Gordts B, Descheemaecker P, Van Landuyt H. Evaluation of a disk diffusion method with cefoxitin (30 μg) for detection of methicillin-resistant *Staphylococcus aureus*. *Eur J Clin Microbiol Infect Dis* 2004; **23**: 389–392.
13. Boubaker IBB, Abbes RB, Abdallah HB *et al*. Evaluation of a cefoxitin disk diffusion test for the routine detection of methicillin-resistant *Staphylococcus aureus*. *Clin Microbiol Infect* 2004; **10**: 762–765.
14. Velasco D, del Mar Tomas M, Cartelle M *et al*. Evaluation of different methods for detecting methicillin (oxacillin) resistance in *Staphylococcus aureus*. *J Antimicrob Chemother* 2005; **55**: 379–382.
15. Swenson JM, Tenover FC, the Cefoxitin Disk Study Group. Results of disk diffusion testing with cefoxitin correlate

with presence of *mecA* in *Staphylococcus* spp. *J Clin Microbiol* 2005; **43**: 3818–3823.
16. Pottumarthy S, Fritsche TR, Jones RN. Evaluation of alternative disk diffusion methods for detecting *mecA*-mediated oxacillin resistance in an international collection of staphylococci: validation report from the SENTRY antimicrobial surveillance program. *Diagn Microbiol Infect Dis* 2005; **51**: 57–62.
17. Frigatto E, Machado A, Pignatari A, Gales AC. Is the cefoxitin disk test reliable enough to detect oxacillin resistance in coagulase-negative staphylococci? *J Clin Microbiol* 2005; **43**: 2028–2029.
18. National Committee for Clinical Laboratory Standards. *Performance standards for antimicrobial disk susceptibility tests*, 8th edn. Approved standard M2-A8. Wayne, PA: NCCLS, 2003.
19. National Committee for Clinical Laboratory Standards. *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically*, 6th edn. Approved standard M7-A6. Wayne, PA: NCCLS, 2003.

RESEARCH NOTE

Investigation of suspected laboratory cross-contamination: interpretation of single smear-negative, positive cultures for *Mycobacterium tuberculosis*

V. J. Cook[1,2], G. Stark[1], D. L. Roscoe[3,4], A. Kwong[3] and R. K. Elwood[1,2]

[1]Division of TB Control, British Columbia Centre for Disease Control, [2]Department of Medicine, University of British Columbia, [3]Department of Pathology and Laboratory Medicine and [4]Department of Pathology and Laboratory Medicine, Vancouver General Hospital, Vancouver, Canada

ABSTRACT

Restriction fragment length polymorphism (RFLP) analysis can be used to assess genetic relatedness of *Mycobacterium tuberculosis* isolates. This study reports a collaborative investigation of false-positive cultures for *M. tuberculosis*, suspected when the DNA fingerprint from an index case matched an epidemiologically improbable source case. RFLP analysis matched fingerprints in ten of 16 cases of suspected laboratory contamination to four separate smear-positive sources that were processed on the same day in the same laboratory. All single smear-negative, positive cultures processed on the same day as smear-positive specimens should be reviewed on a case-by-case basis to identify possible false-positive cultures.

Corresponding author and reprint requests: V. J. Cook, TB Control, BC Centre for Disease Control, 655 West 12th Avenue, Vancouver, BC V5Z 4R4, Canada
E-mail: victoria.cook@bccdc.ca

Keywords Cross-contamination, DNA fingerprints, false-positive cultures, *Mycobacterium tuberculosis*, restriction fragment length polymorphism analysis, tuberculosis

Original Submission: 21 October 2005; **Revised Submission:** 22 February 2006; **Accepted:** 2 April 2006

Clin Microbiol Infect 2006; 12: 1042–1045
10.1111/j.1469-0691.2006.01517.x

A clinical diagnosis of tuberculosis (TB) is, ideally, confirmed with a positive culture. Even in cases without symptoms or with a normal chest radiograph, a positive culture constitutes an active case in need of treatment, as colonisation with *Mycobacterium tuberculosis* is not thought to occur. Recent advances have improved the diagnosis of TB, but have been associated with a parallel rise in the number of reports of false-positive cultures [1,2]. Laboratory cross-contamination is a well-documented source of false-positive cultures, which have important clinical and public health consequences [3–10]. Diagnostic criteria for suspected laboratory cross-contamination with *M. tuberculosis* have been published [11,12], and the recent rise in the number of reports is probably related to the ease with which these incidents can be confirmed by molecular epidemiological studies [13,14]. The present report describes a retrospective collaborative investigation of four clusters of false-positive TB cultures caused by probable laboratory cross-contamination. The investigation was initiated when the restriction fragment length polymorphism (RFLP) cluster results for a case could not be explained by epidemiological evaluation.

Between December 2001 and January 2003, 84 *M. tuberculosis* isolates from 44 patients were identified by laboratory culture according to published guidelines [15,16]. Laboratory contamination was considered if patients had a single